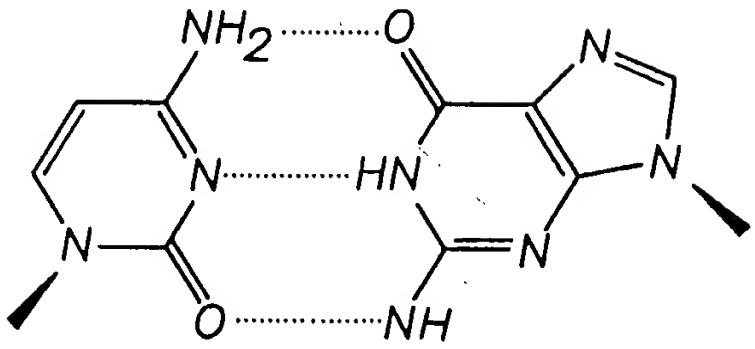
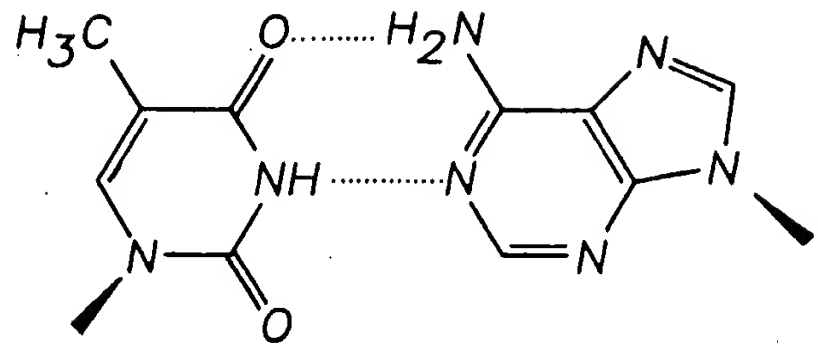
FIG. 1A



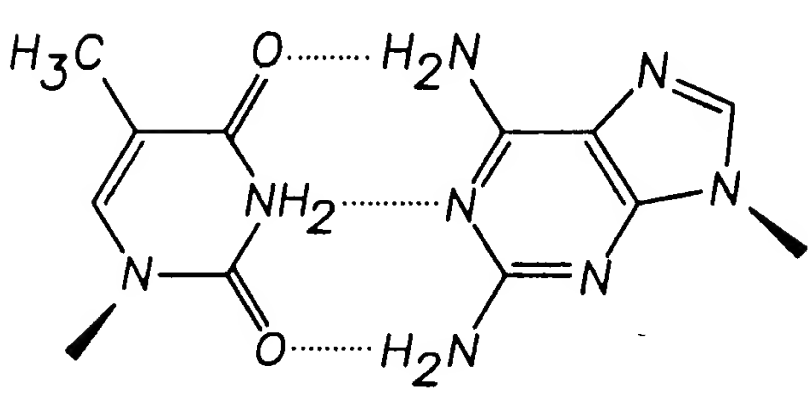
C • G

FIG. 1B



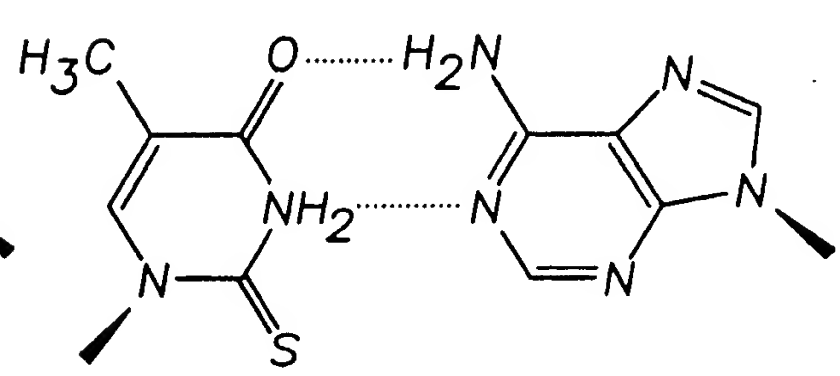
T • A

FIG. 1C



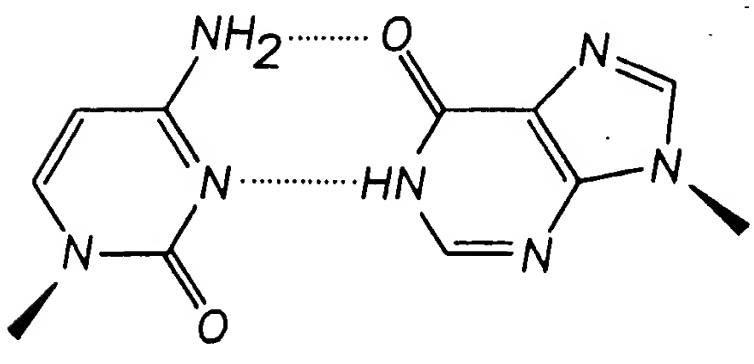
T • 2–aminoA

FIG. 1D



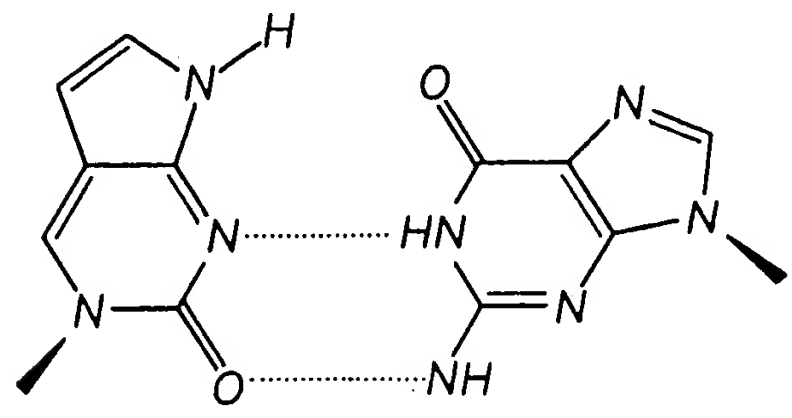
2–thioT • A

FIC.1E



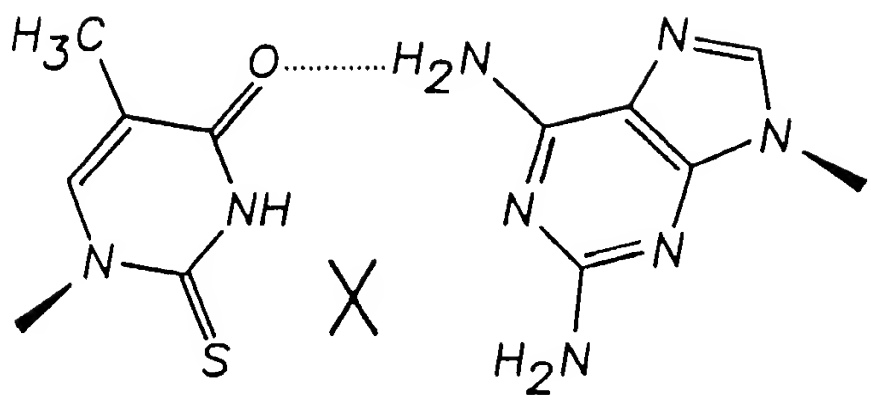
C • I

FIG. 1F



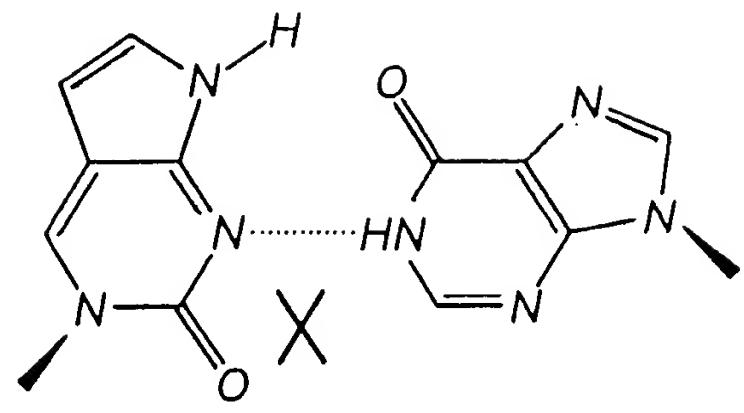
PyrroloPyr • G

FIG. 1G



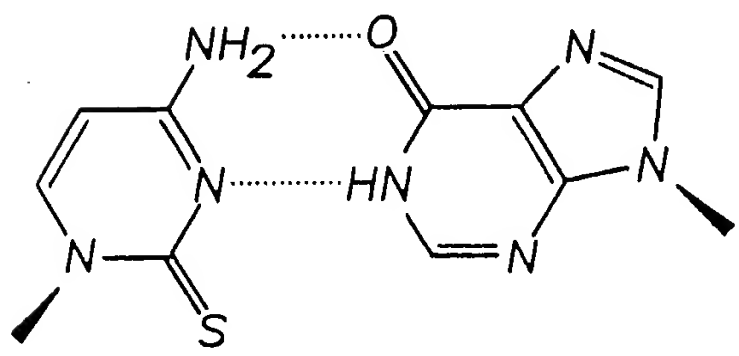
2-thioT•2-aminoA

FIG. 1H



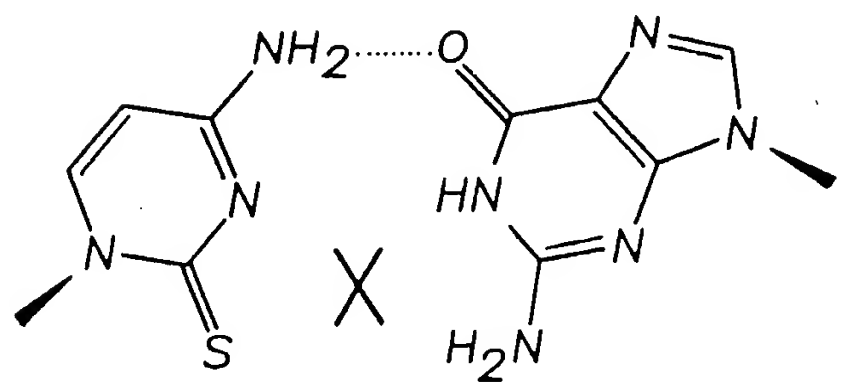
PyrroloPyr•I

FIG. 1I



2-thioC•I

FIG. 1J



2-thioC•G

FIG. 2A

```
5′-CGATAGGCTCTG →
3′-GCTATCCGAGACCCTGACTTGACACCTGTT-5′
```

FIG. 2B

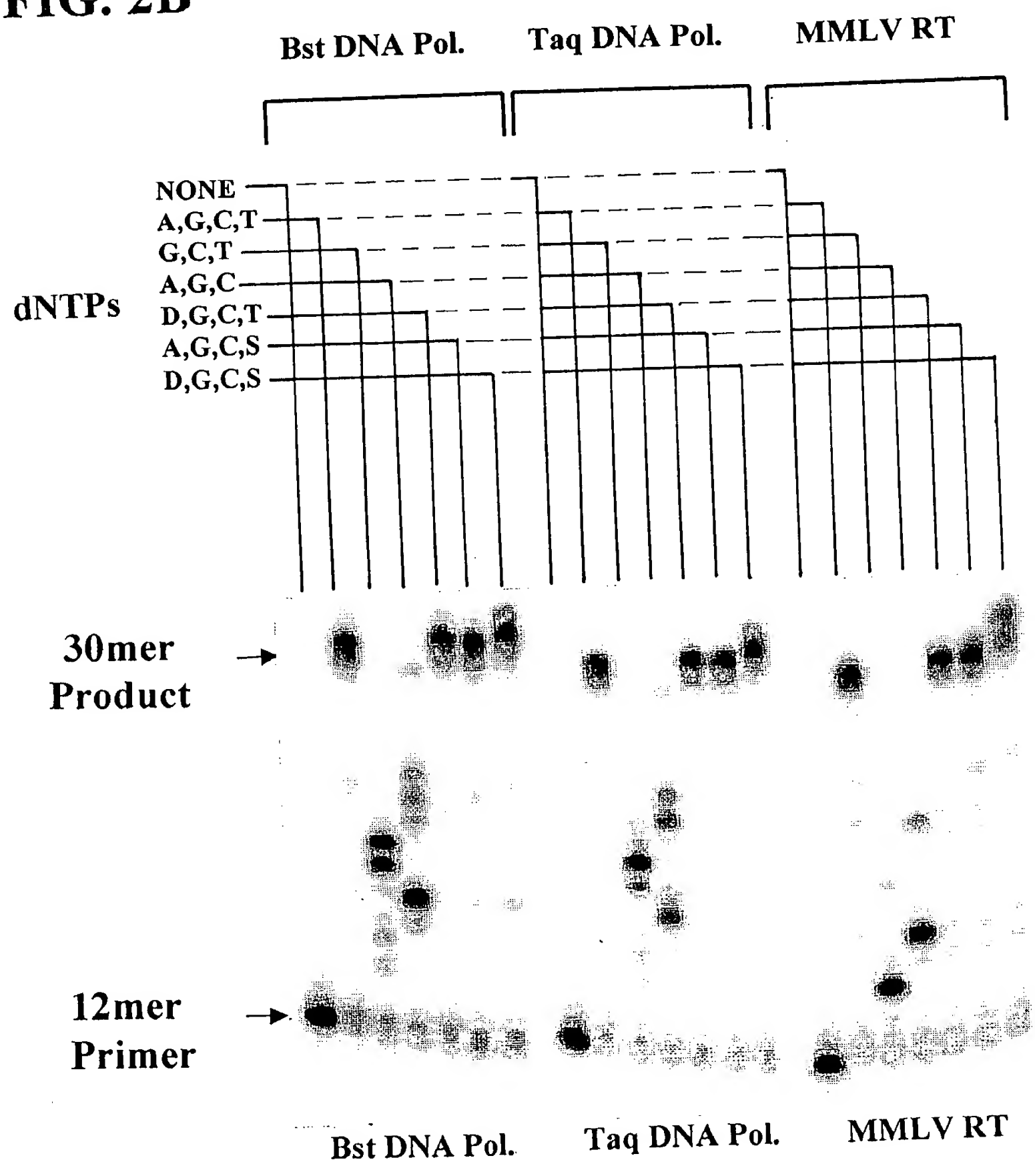


FIG. 3A

5′-GACTGA→

3′-GCTATCCGAGACACTGACTTGACACCTGTT-5′

FIG. 3B

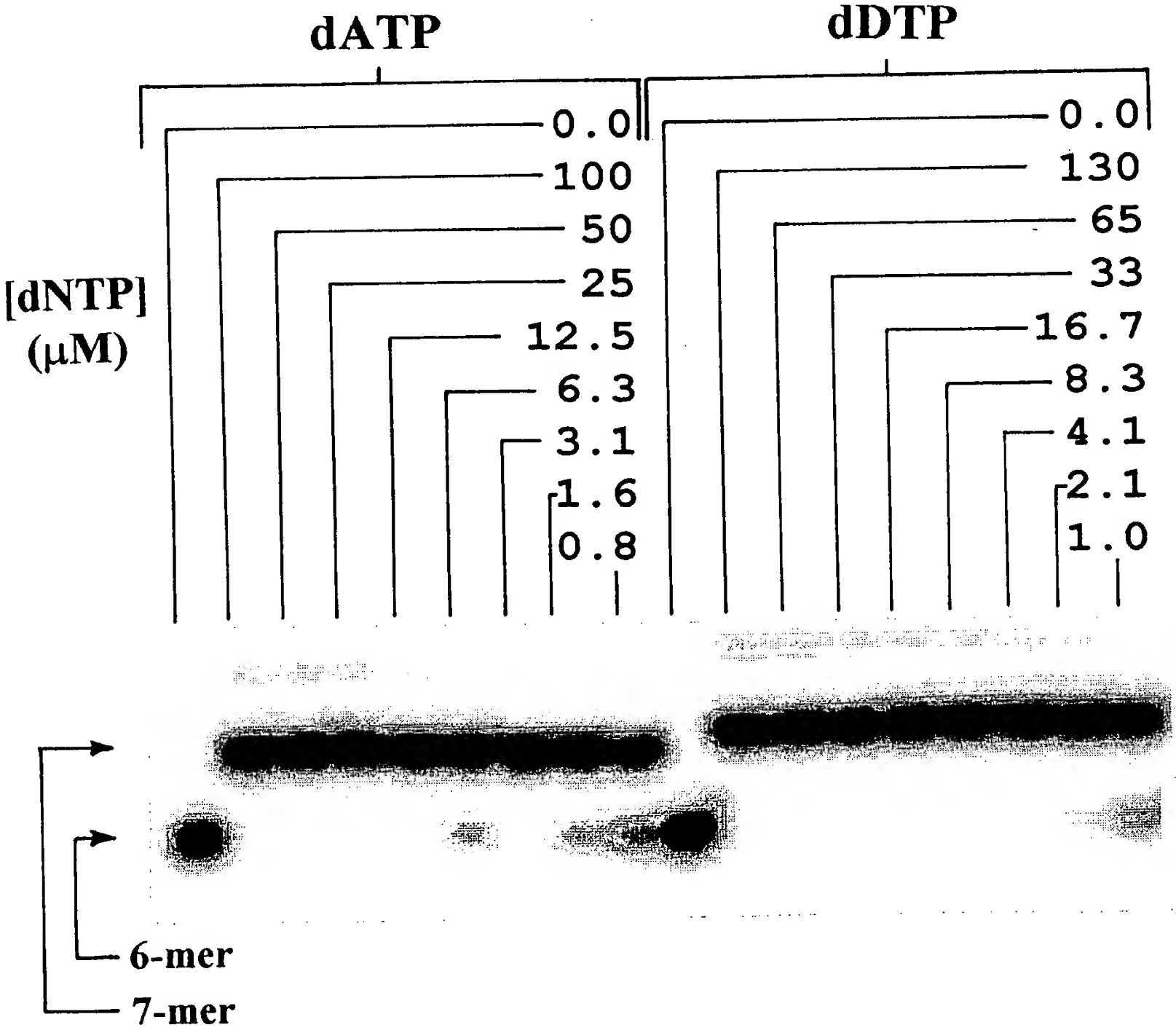


FIG. 3C

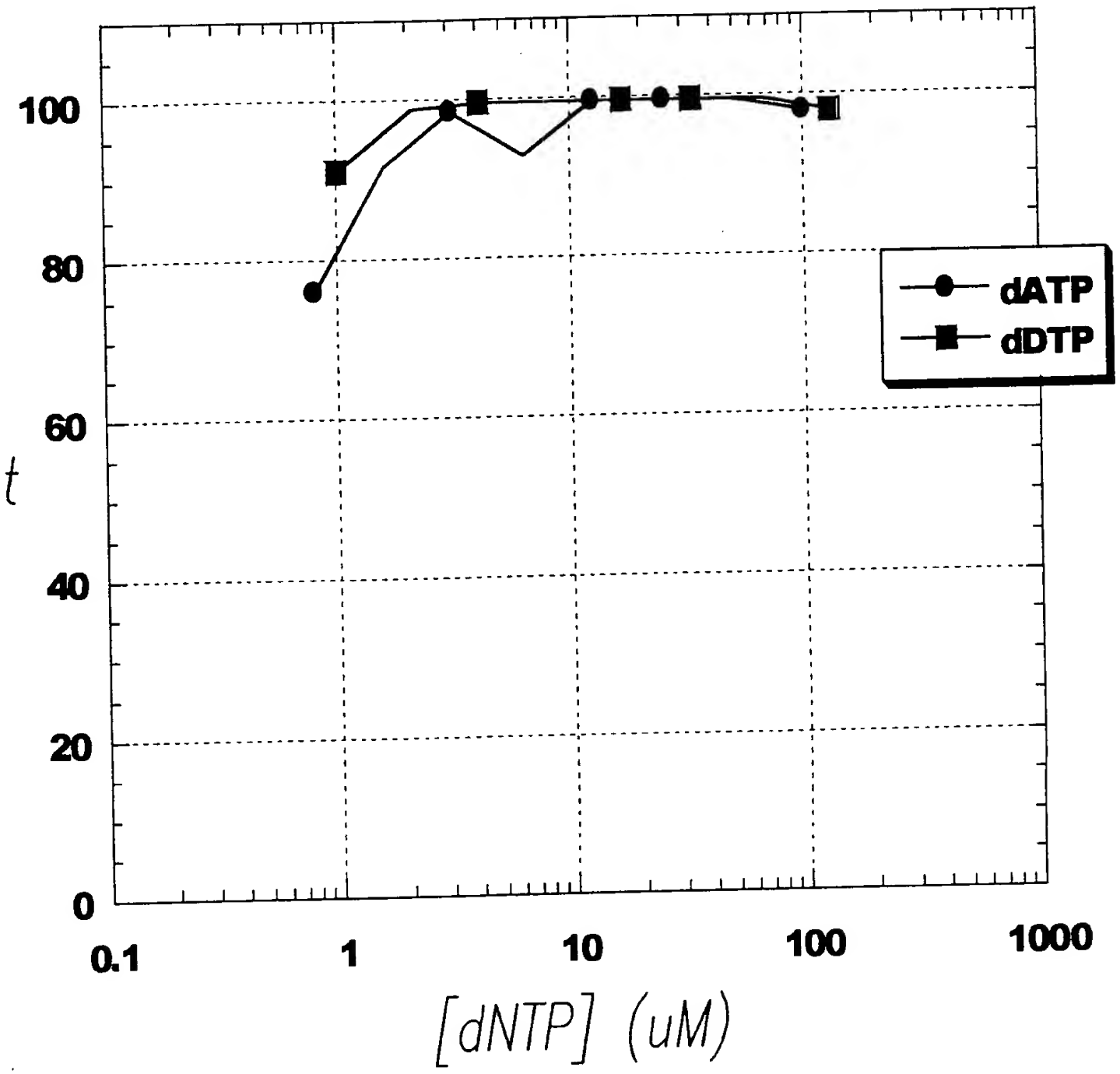


FIG. 4A

5′-GCTCTG →
3′-GCTATCCGAGACACTGACTTGACACCTGTT-5′

FIG. 4B

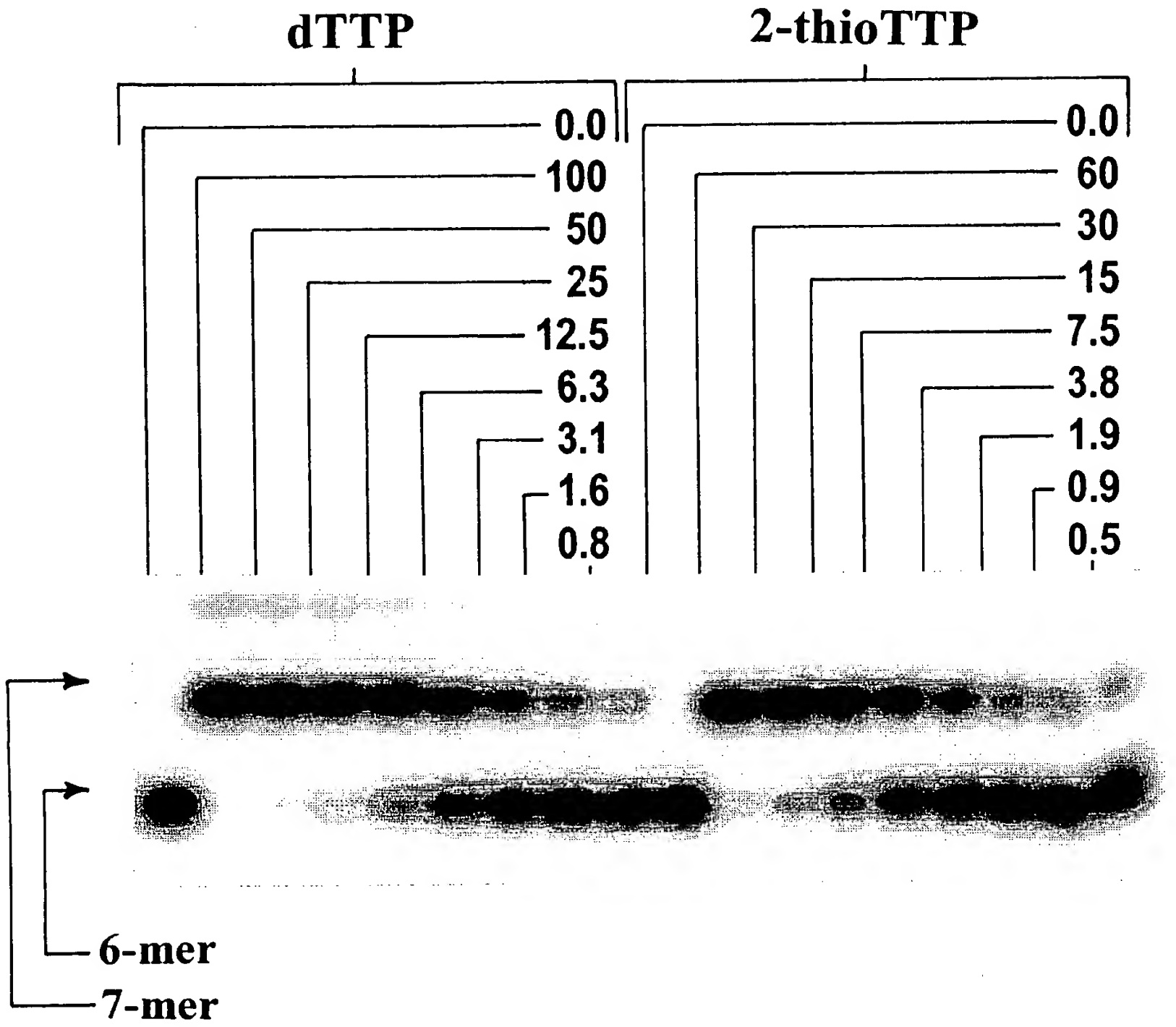


FIG. 4C

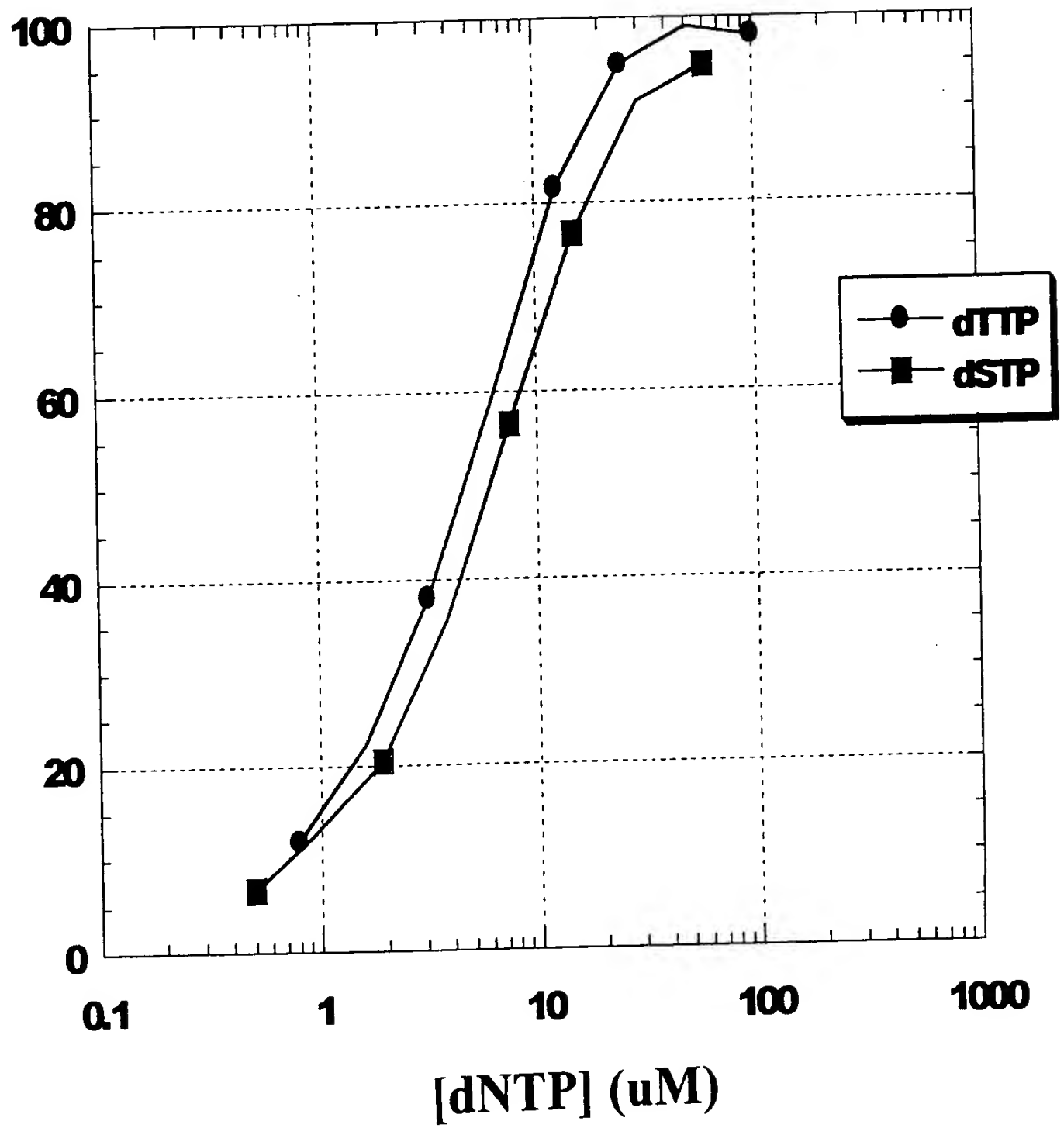


Fig. 5A

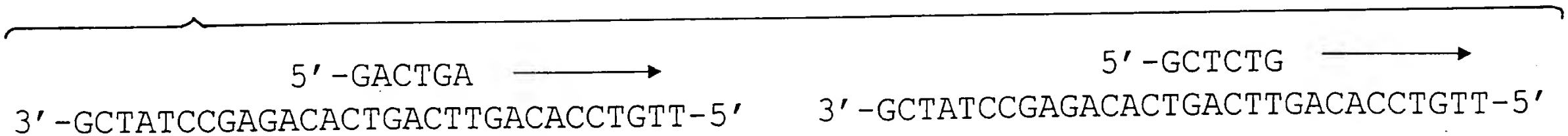


FIG. 5C

Extension Nucleotide	X-mer	Predicted m/z (Positive Ion)*	Measured m/z (Positive Ion)	Predicted Δ m/z	Measured Δ m/z
None	GACTGA	1816.3	nd	--	--
dATP	GACTGAA	2129.5	2130.2	+15.0	+15.8
dDTP	GACTGAD	2144.5	2146.0		
None	GCTCTG	1783.2	1785.4 ± 0.2	--	--
dTTP	GCTCTGT	2087.4	2089.7	+16.0	+16.4
d-2-thio-TTP	GCTCTGS	2103.4	2106.1		

Fig. 5B-1

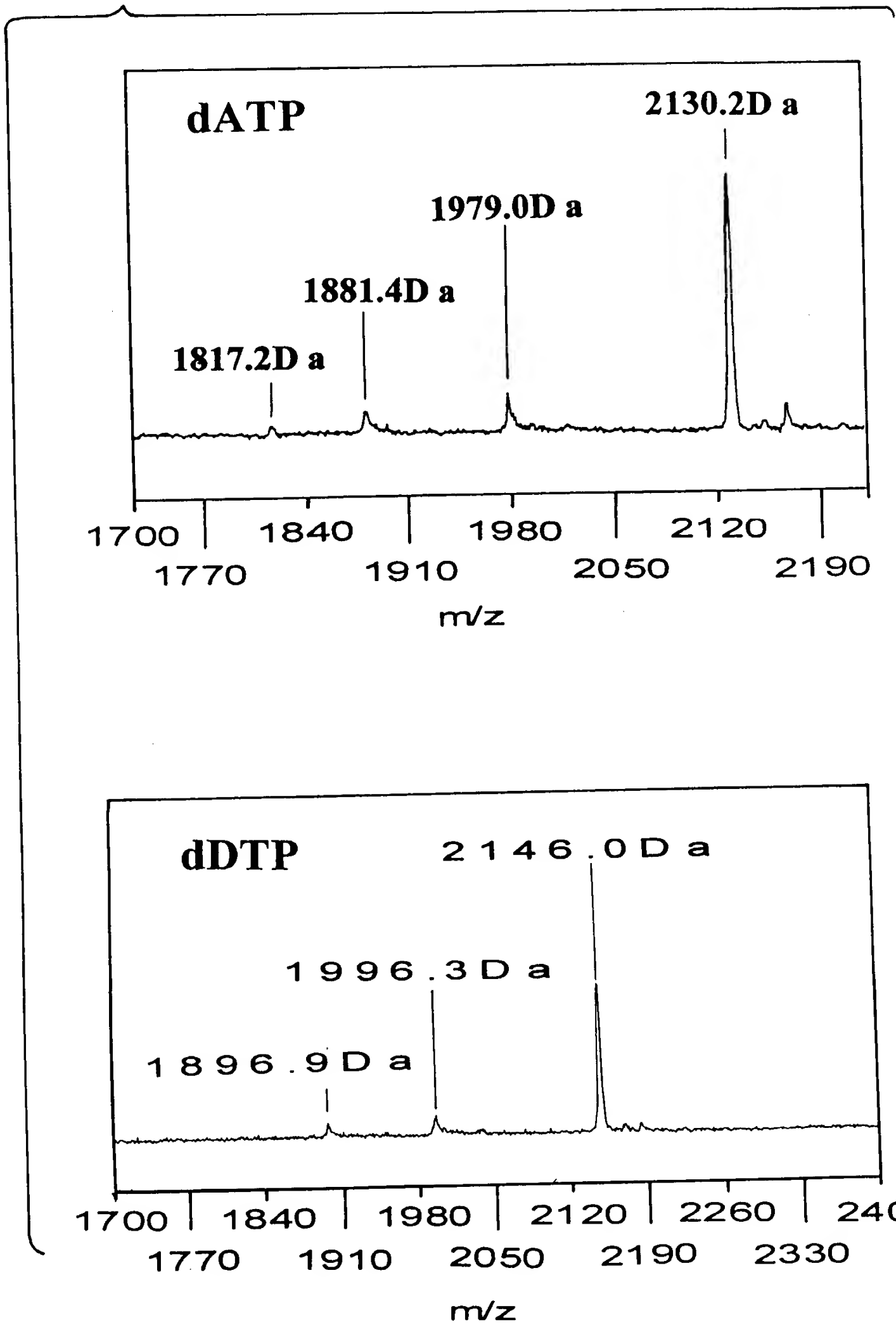


Fig. 5B-2

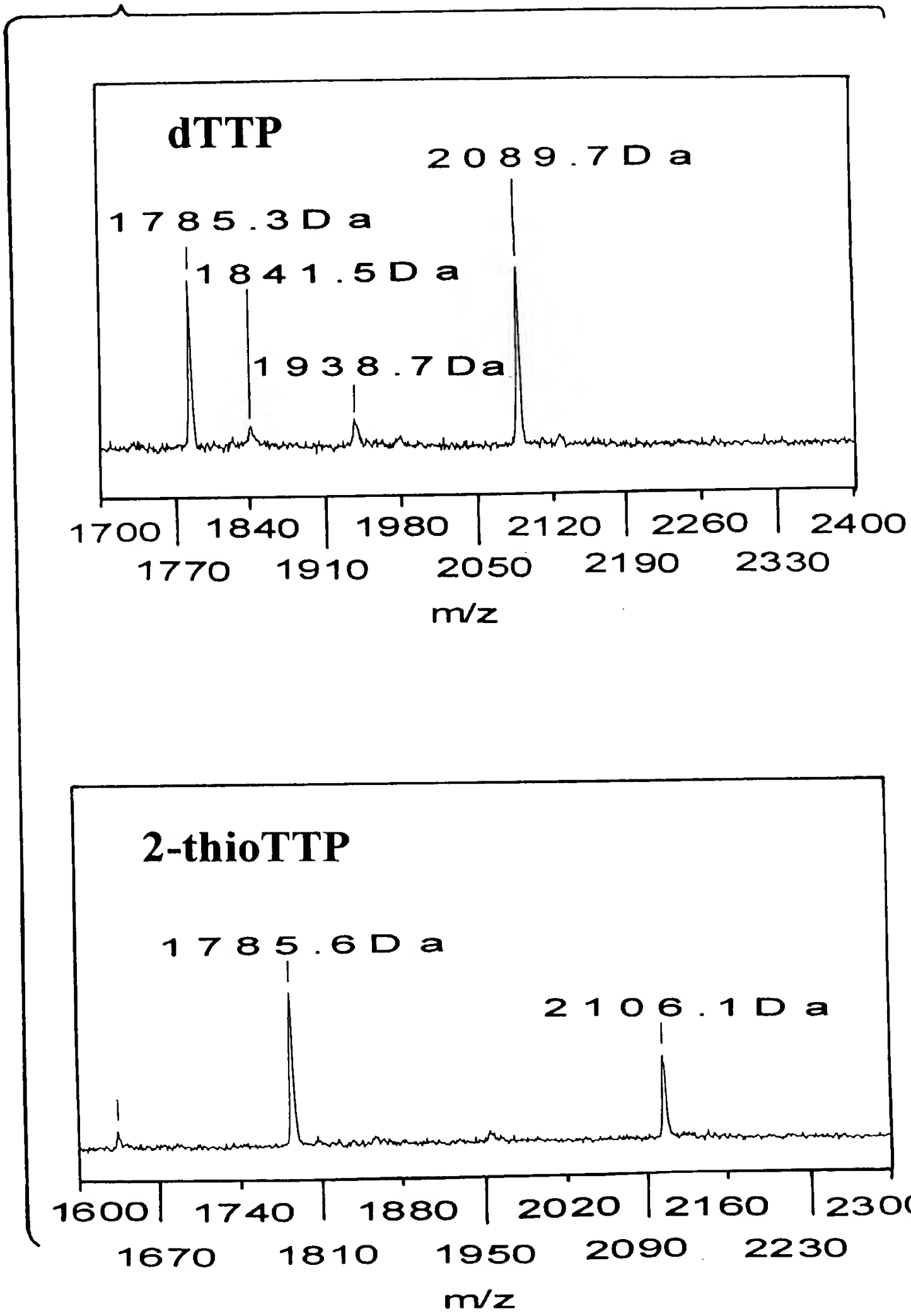


FIG. 6

5′-CTATCCGATCCATC→
3′-GATAGGCTAGGTAGTTCAAGTCAGAGC**TTT**GTCAGAGTTGT**AAA**CAGGTGTCGCAT**p**-5′

↓ **dNTPs**
Bst DNA Polymerase

5′-CTATCCGATCCATCaagttcagtctcg**aaa**cagtctcaaca**ttt**gtccacagcgta
3′-GATAGGCTAGGTAGTTCAAGTCAGAGC**TTT**GTCAGAGTTGT**AAA**CAGGTGTCGCAT**p**-5′ ←

↓ **λ Exonuclease**

5′-CTATCCGATCCATCaagttcagtctcg**aaa**cagtctcaaca**ttt**gtccacagcgta-3′

+ pA, pG, pC pT

FIG. 7

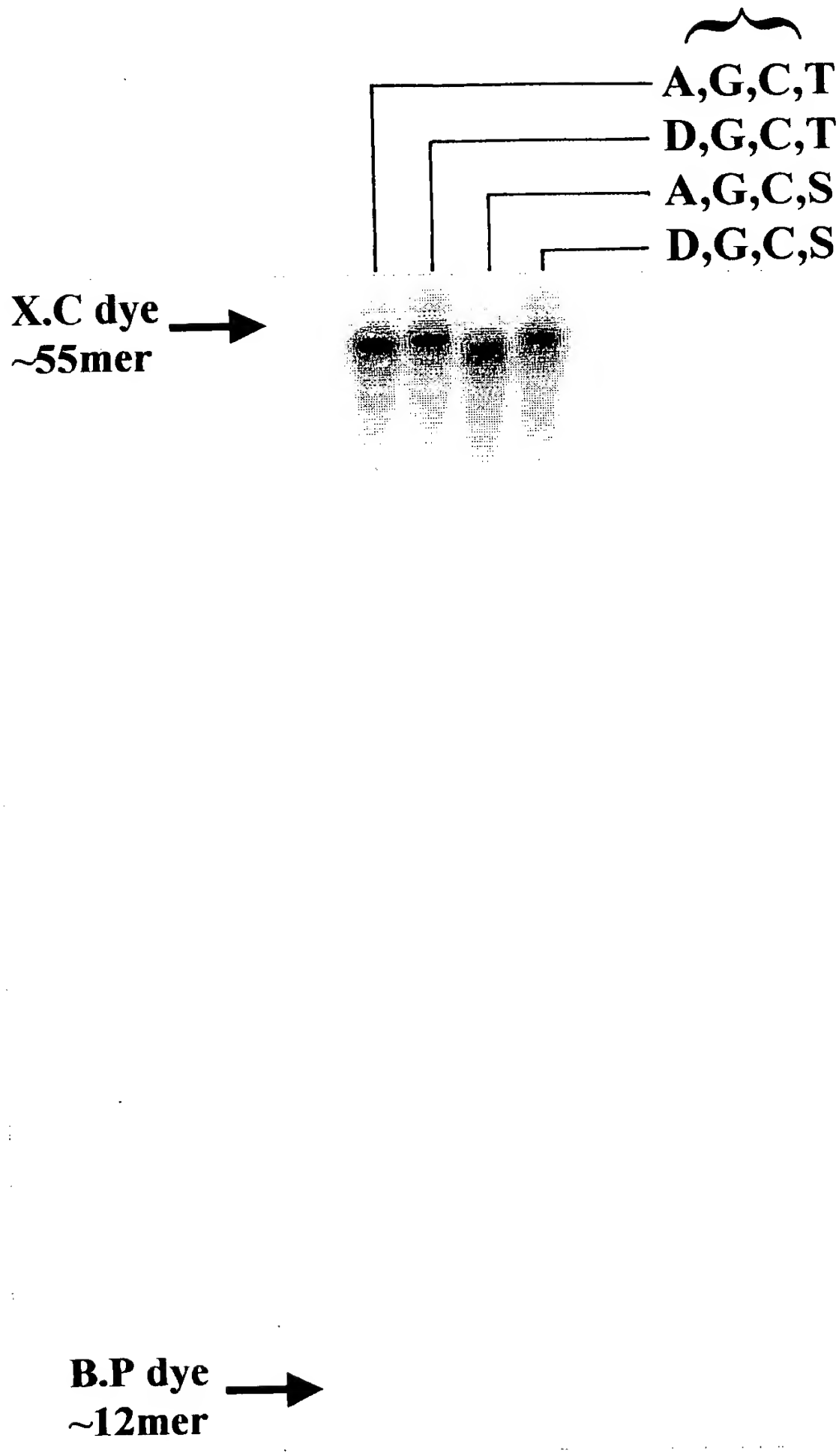


FIG. 8A

HP21AT

ΔG° = -12.3 kcal/mole at 37 °C
Δ H° = -82.5 kcal/mole
Δ S°= -226.3 cal/ (°K·mol)
Tm = 91.4°C

```
          10        20
5'-CTATCCGATCCATCAA          G
                   GT TCAGTCT C A
                   |||||||||| |
                   CAAGTCAGAG A
3'-ATGCGACACCTGTTTA          A
       50        40        30
```

HP21DS

```
          10        20
5'-CTATCCGATCCATCDD          G
                   GS SCDGSCS C D
                   |..|.|.|.| |
                   CDDGSCDGDG D
3'-DSGCGDCDCCSGSSSD          D
       50        40        30
```

13/26

FIG. 8B

HP26AT

Δ G° = -3.8 kcal/mole at 37 °C
Δ H° = -41.2 kcal/mole
Δ S°= -120.5 cal/ (°K·mol)
Tm = 68.8°C

```
            10          20
5′-CTATCCGATCCATCAA    C   T    G
                     GTT AG CTC A
                     ||| || ||| 
                     CAA TC GAG A
3′-ATGCGACACCTGTTTA    C   T    A
        50          40          30
```

HP26DS

```
            10          20
5′-CTATCCGATCCATCDD    C   S    G
                     GSS DG CSC D
                     |.. .| |.|
                     CDD SC GDG D
3′-DSGCGDCDCCSGSSSD    C   S    D
        50          40          30
```

FIG. 8C

HP28AT

Δ G° = 0.1 kcal/mole at 37 °C
Δ H° = -27.4 kcal/mole
Δ S°= -88.6 cal/ (°K·mol)
Tm = 36.1°C

```
          10        20
5'-CTATCCGATCCATCAA   C  T  CG
                   GT T AG CT  A
                   ||| || ||
                   CAA TC GA  A
3'-ATGCGACACCTGTTTA   C  T  CA
        50        40        30
```

HP28DS

```
          10        20
5'-CTATCCGATCCATCDD   C  S  CG
                   GS S DG CS  D
                   |.. .| |.
                   CDD SC GD  D
3'-DSGCGDCDCCSGSSSD   C  S  CD
        50        40        30
```

FIG. 9A

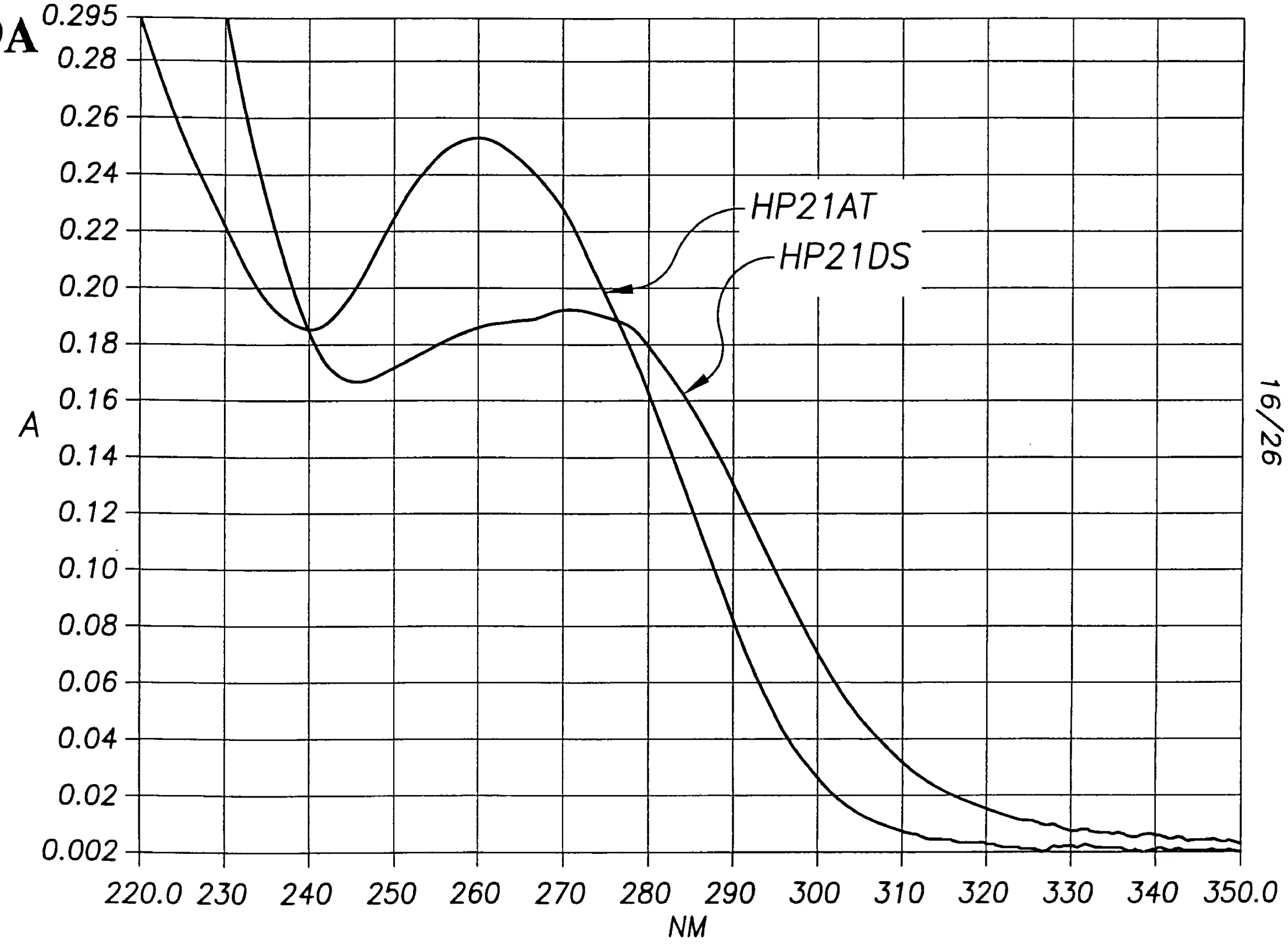


FIG. 9B

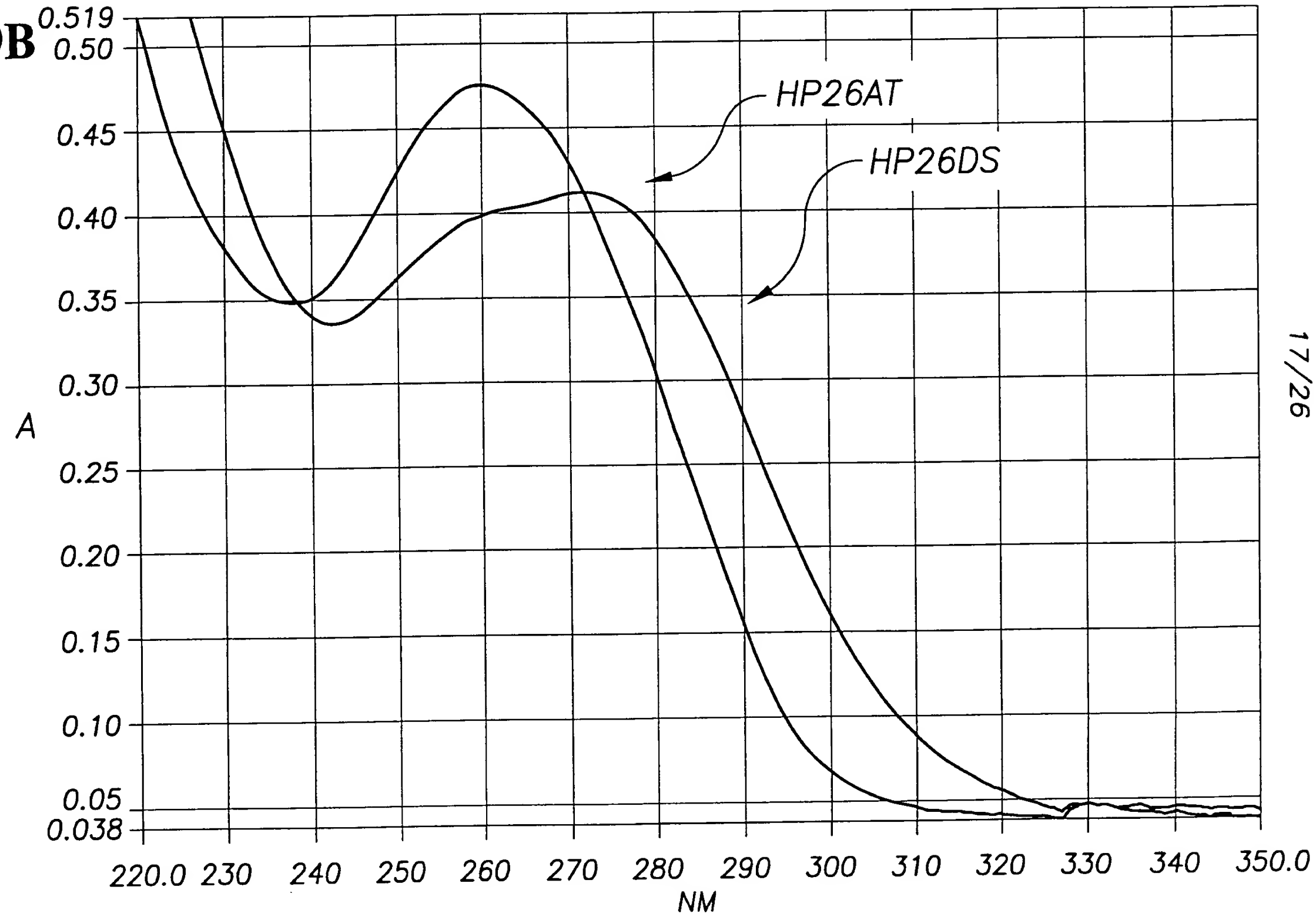


FIG. 9C

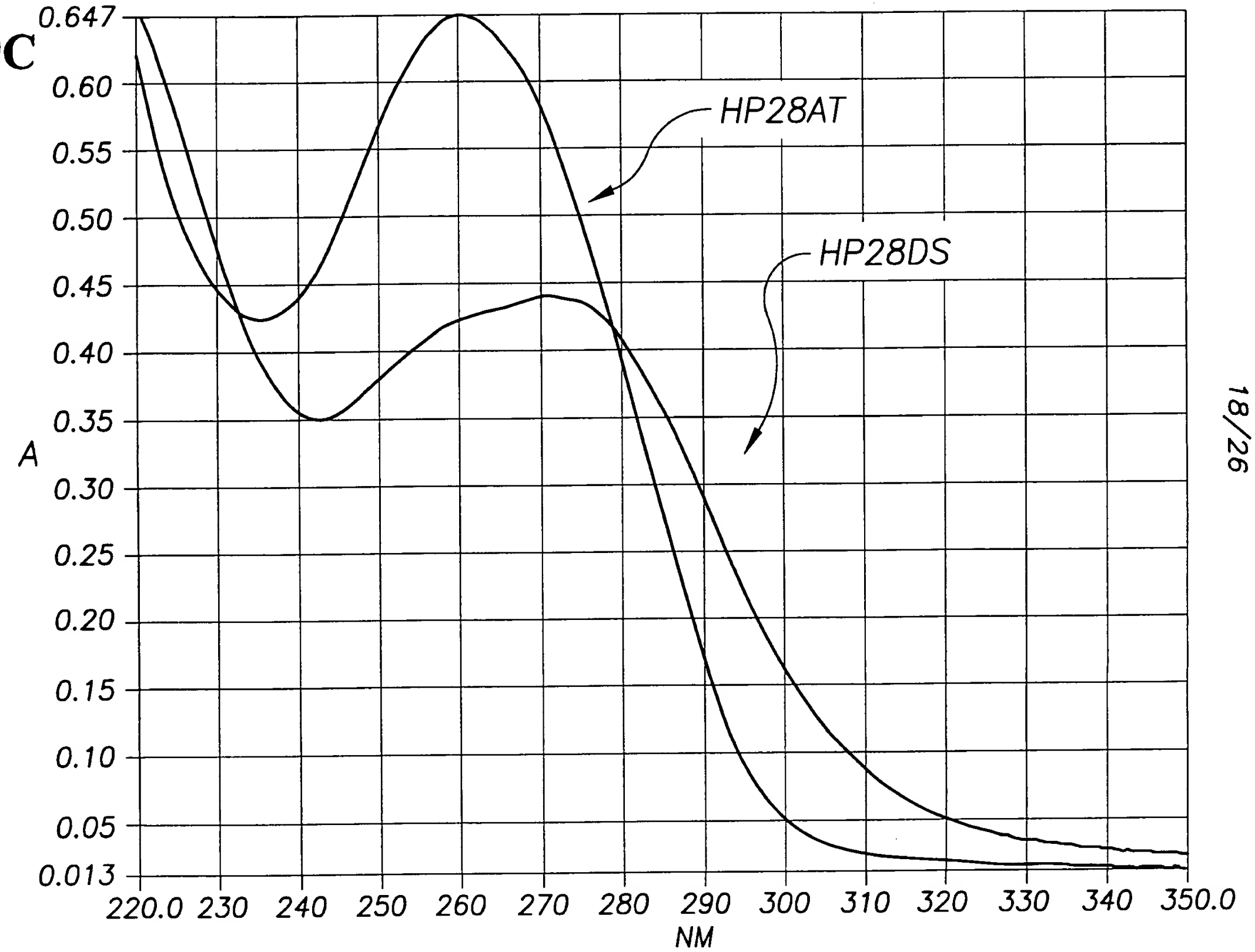
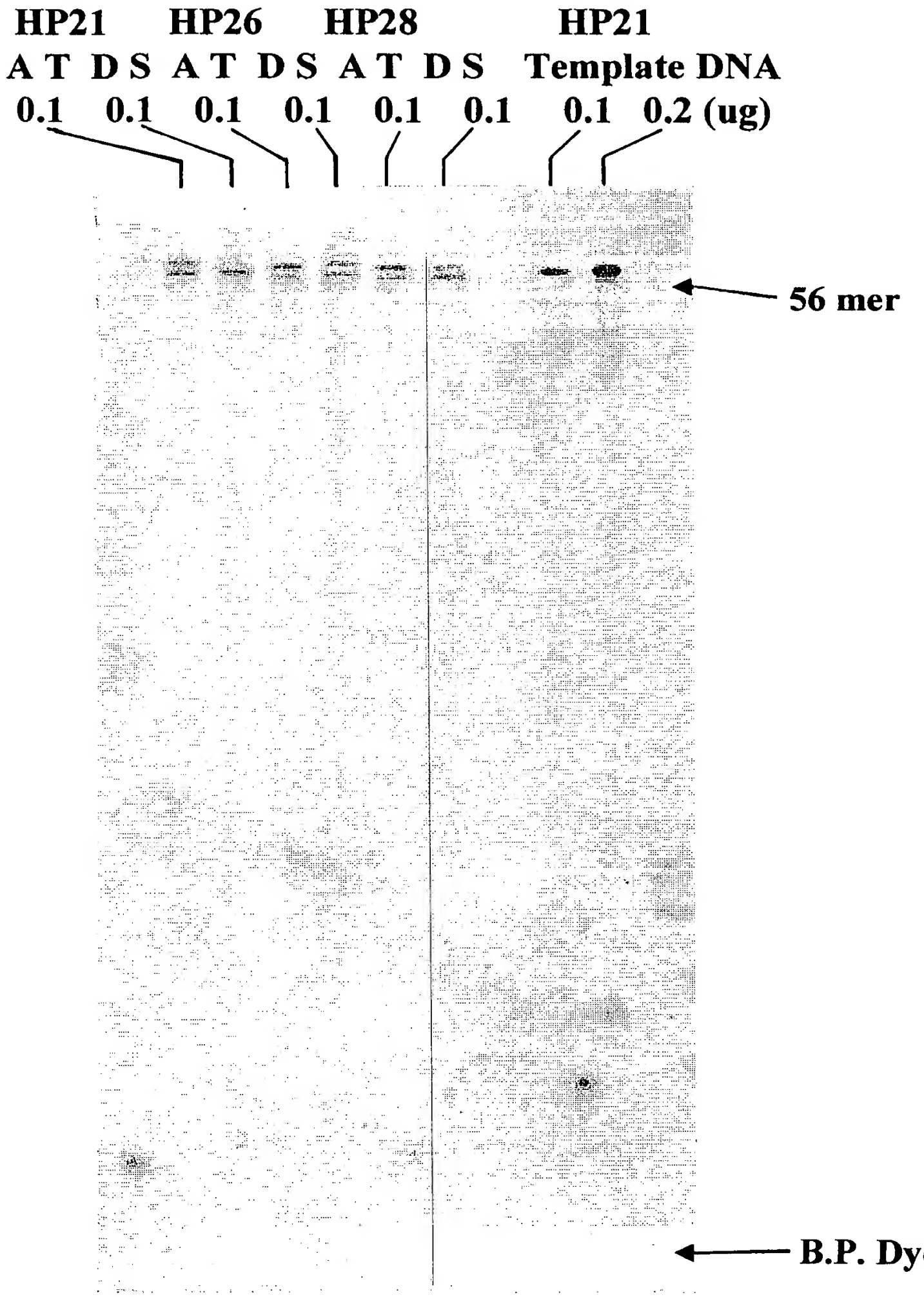


FIG. 10



10% 7M Urea PAGE

FIG. 11A

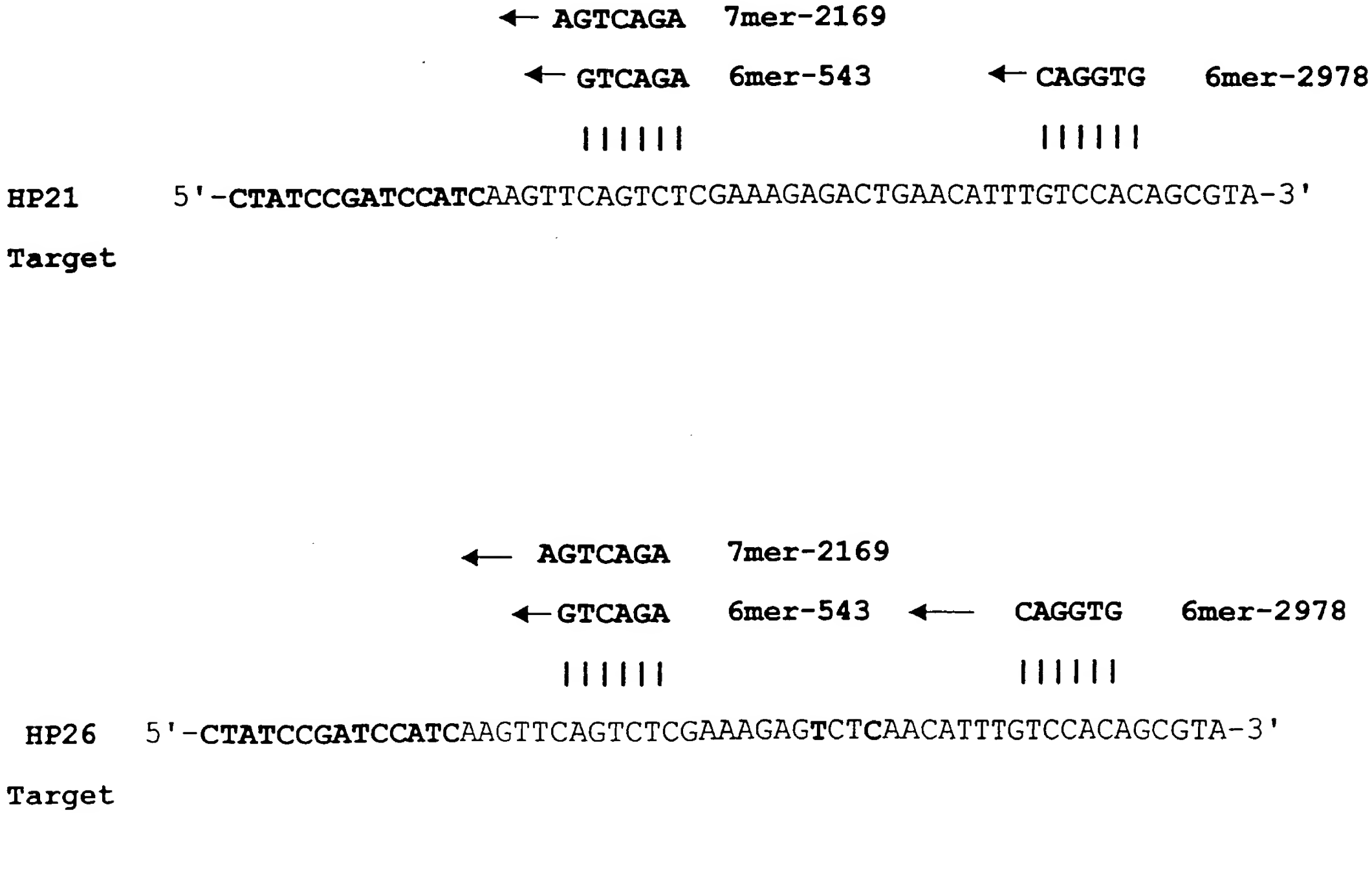


FIG. 11B

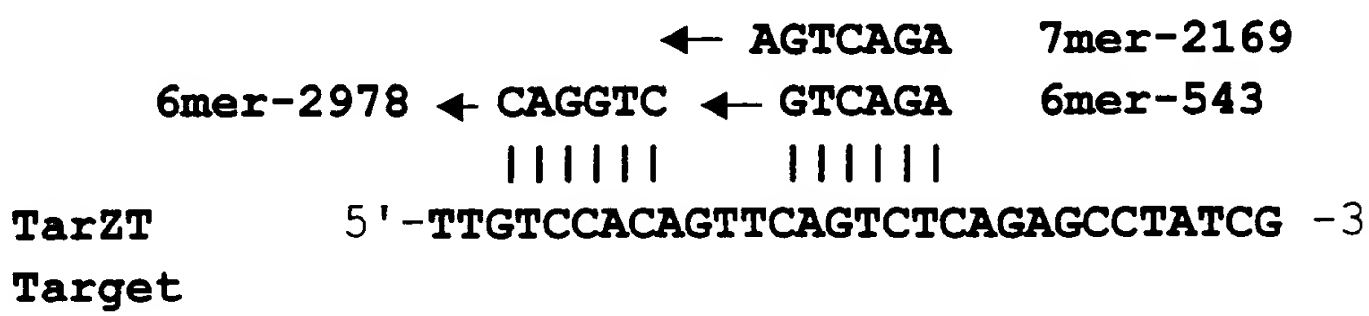


FIG. 12A

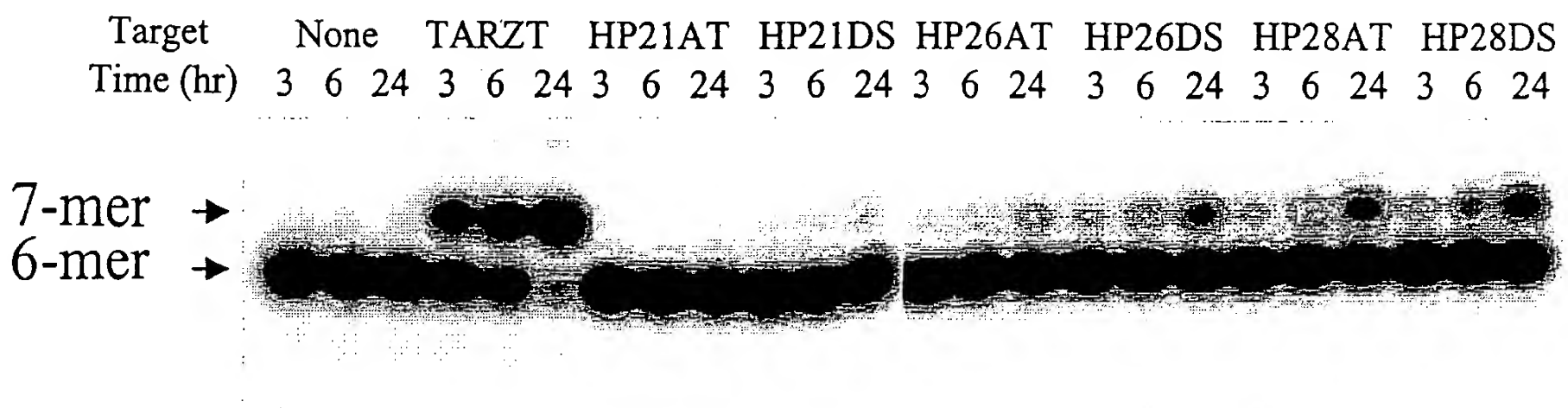
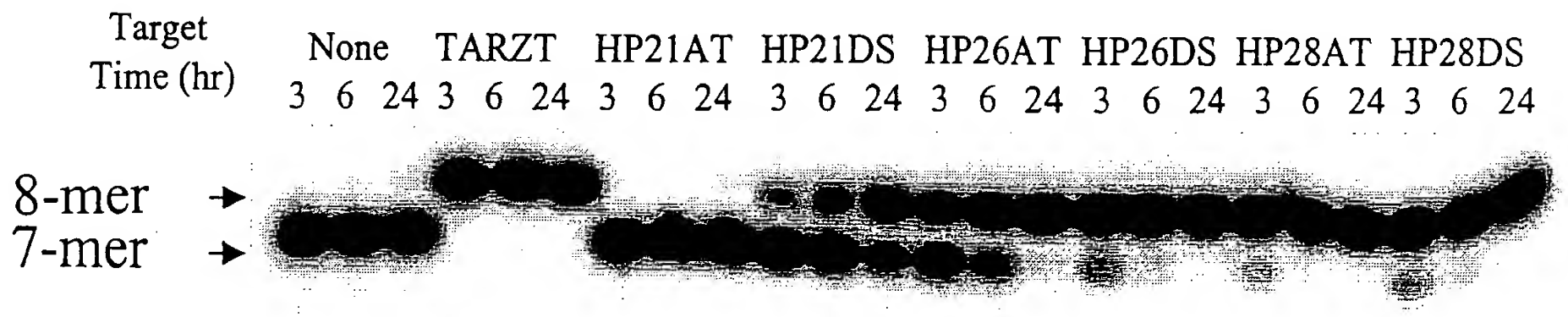


FIG. 12B



6-mer 2978

FIG. 12C

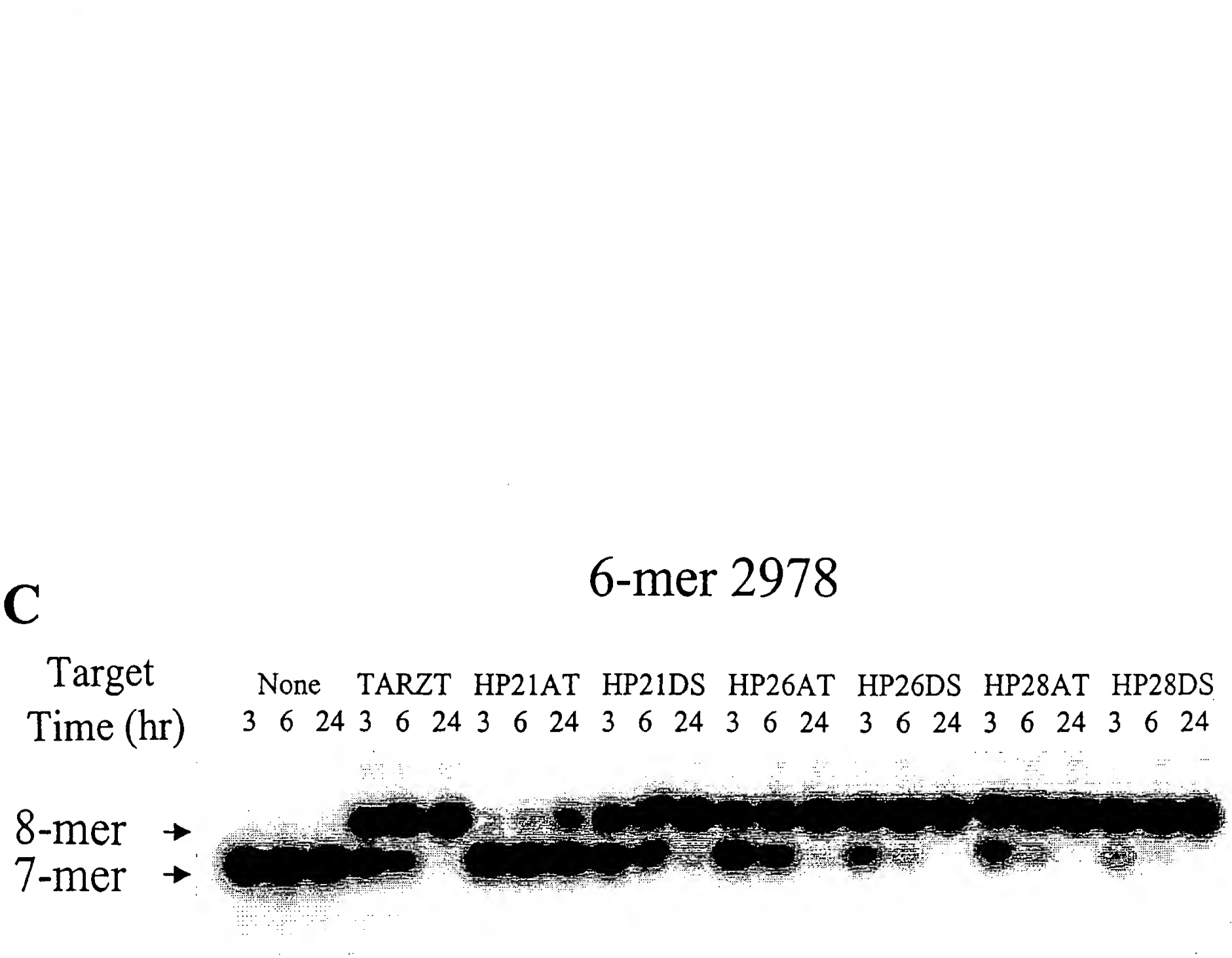


FIG. 13A

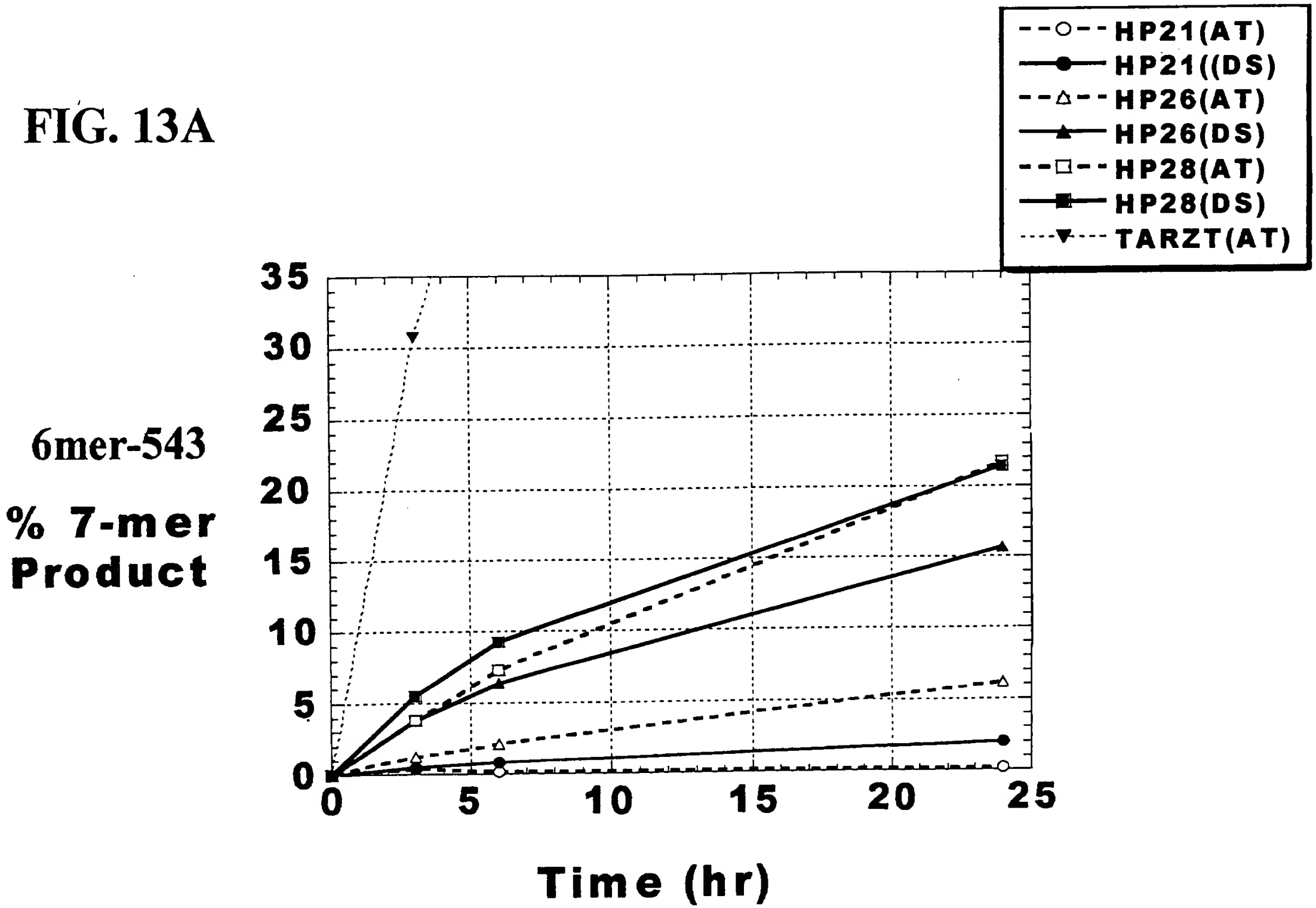


FIG. 13B

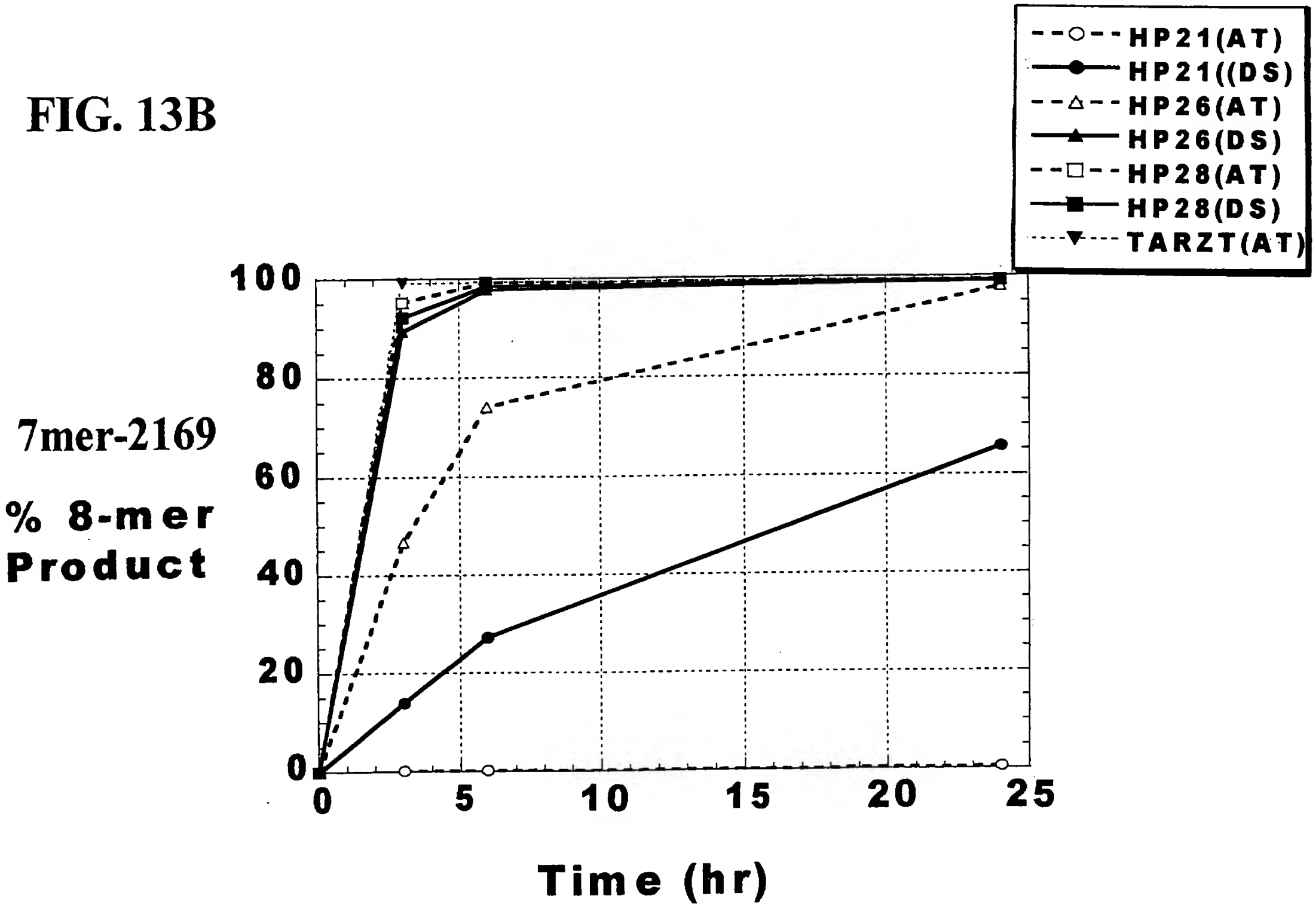


FIG. 13C